FORM PTO-1390 (REV. 12-2001) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 0380-P02810US0

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 10/049526 not yet assigned

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/03054	08 August 2000	11 August 1999

TITLE OF INVENTION: SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE

APPLICANT(S) FOR DO/EO/US: VADGAMA, Pankaj

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C 371(f)) The submission must include items (5), (6), (9) and (21) indicated below
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31)
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a [ ] is attached hereto (required only if not communicated by the International Bureau).
   - b [X] has been communicated by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a [ ] is attached hereto.
   - b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4)
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a [ ] are attached hereto (required only if not communicated by the International Bureau).
   - b [ ] have been communicated by the International Bureau.
   - c [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment
14. [ ] A SECOND or SUBSEQUENT preliminary amendment
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:

Copy of claims 1-22, as amended under Article 34.

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U S APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
not yet assigned 10/049326	PCT/GB00/03054	0380-P02810US0

21. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . $740.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) . . . $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	S 890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e))	S 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	17 - 20 =	0	x $18.00	S 0
Independent claims	1 - 3 =	0	x $84.00	S 0
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	S 0
TOTAL OF ABOVE CALCULATIONS =				S 1,020.00
[X] Applicant claims small entity status See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	S 510.00
SUBTOTAL =				S 510.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				S 0
TOTAL NATIONAL FEE =				S 510.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				S 0
TOTAL FEES ENCLOSED =				S 510.00
			Amount to be refunded:	$
			charged:	$

a. [X] A check in the amount of $ 510.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-1406. A duplicate copy of this sheet is enclosed.

d [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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THE UNITED STATES PATENT AND TRADEMARK OFFICE

United States Serial No.	:	Not Yet Assigned
International Application No.	:	PCT/GB00/03054
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Inventor(s)	:	Pankaj Vadgama
Title	:	SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE

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Assistant Commissioner
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PRELIMINARY AMENDMENT

Dear Sir:

Before calculation of the filing fee, please amend, as follows, the indicated claims of the above-identified patent application, which claims incorporate the amendments under Article 34 filed in the corresponding international patent application:

3. (Amended) The sensor device of claim 1, wherein the wire electrode sensor is formed from a noble metal.

6. (Amended) The sensor device of claim 1, wherein the wire has an outer diameter of 50 to 150μm.

7. (Amended) The sensor device of claim 1, wherein the enzyme is an oxidase or dehydrogenase enzyme.

9. (Amended) The sensor device of claim 1, wherein the enzyme is retained in the cavities by cross-linking.

12. (Amended) The sensor device of claim 1, wherein the electrode further comprises a coating over the electrode and enzyme present in the cavities.

16. (Amended) The sensor device of claim 1, wherein the cavities are produced by micromachining with an ion beam or a laser.

17. (Amended) The sensor device of claim 1, wherein the surface or the wire electrode is covered by a coating of insulating material.

Cancel claims 11, 19-22.

REMARKS

The purpose of this Preliminary Amendment is to delete multiple claims dependencies and to cancel claims which, due to their form, do not comply with current U.S. Patent and Trademark Office practice.

A marked-up version of amended claims 3, 6, 7, 9, 12, 16, and 17 is attached hereto.

Entry of the present amendments is hereby requested. These claim amendments do not introduce new matter into the application.

Dated: February 11, 2002

Patrick J. Hagan
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Attorney for Applicants

MARKED-UP COPY OF THE AMENDED CLAIMS

3. (Amended) The sensor device of claim 1 [or claim 2], wherein the wire electrode sensor is formed from a noble metal.

6. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the wire has an outer diameter of 50 to 150μm.

7. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the enzyme is an oxidase or dehydrogenase enzyme.

9. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the enzyme is retained in the cavities by cross-linking.

12. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the electrode further comprises a coating over the electrode and enzyme present in the cavities.

16. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the cavities are produced by micromachining with an ion beam or a laser.

17. (Amended) The sensor device of claim 1 [any one of the preceding claims], wherein the surface or the wire electrode is covered by a coating of insulating material.

SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE.

This invention relates to improved sensor devices and methods for their analytical use, and more particularly to improved forms of enzyme electrodes.

There is an increasing need for procedures and devices which can enable the presence and amount of particular components (analytes) in biological media to be measured without having to rely on taking samples periodically and taking them away to be analysed in a laboratory. Such old "sampling" procedures - though usually accurate - are too slow and involve a significant delay in obtaining the result of the measurement, and in many circumstances this delay can be inconvenient and even dangerous to a subject.

Instead, it is very desirable to have a continuing monitoring procedure can be carried and used to detect changes as near as possible to the moment at which they occur, especially if the changes are abrupt or fluctuating. This is important because conditions in some biological environments, and especially conditions in vivo, can change unexpectedly and quickly -- and some measurements may be useful as indications of progress or even critically important as vital indication or warning of a need for speedy remedial action.

Especial interest exists in measuring glucose levels in body tissue or body fluids, as glucose is vital for life and its level is greatly affected by some conditions, for example diabetes mellitus. Other analytes, e.g. drugs and metabolic products, are also of comparable interest.

For a continuous monitoring system, it is very desirable to implant a sensor device in the biological environment or medium, and especially an in vivo environment, so that parameters of a living environment or process can be made. Indeed, the only routinely usable monitoring system for an ambulatory diabetic would be either a portable or an in vivo device.

A variety of such implant devices have been proposed, but their success and applicability vary considerably. The devices or approaches suggested hitherto could, in principle, eventually allow for reliable monitoring but they suffer from various deficiencies and are not entirely satisfactory. For example, many can lack sensitivity or specificity and, as results are often plotted as graphs, show difficulty in making true measurements independently of base-line or response slope variability in such graphs. Further, in some cases it can be impossible to arrive at meaningful in vivo data without in vivo calibration, particularly in a tissue matrix, and the implant may even provoke tissue rejection.

It has been proposed to meet some of these needs by inserting into living tissue a sensor device substantially in the form of a needle incorporating a sensor electrode. Such an electrode may be used in conjunction with a reference electrode - which may be combined with the sensing electrode in the sensor device or may be used separately from it, for example on the skin of the subject.

Such sensors commonly use an active sensor electrode in conjunction with an enzyme, so that the sensor device can be made to respond to selected analyte species which would in themselves be inactive at the electrode and so would not be detected by it, e.g. glucose oxidase for measuring glucose content. Likewise, various forms of coating materials or membranes (permeable or permselective) are commonly proposed for regulating the access of analyte to the active electrode or reducing interference from other compounds which could interfere with the effectiveness of the measurements of the desired analyte if they reach the electrode surface.

It has been proposed to use fine wires of an appropriate electrode material, bare or appropriately coated, compatible with the environment in which it is to be inserted. Even the smallest sensor devices proposed so far

have been found to have several difficulties in use, which it would be very desirable to overcome, for example:-

(1) Present devices can be too large for easy use (e.g. they may cause undue discomfort to the subject) so greater miniaturisation is desirable to reduce the effect the implanted sensor may have on the subject's behaviour and tolerance of the implant. Also it is desirable to achieve a size of device which allows for an adequate balance between rigidity and flexibility, so that the device is more durable and more easily implanted.

(2) Many known devices employing any coating on the electrode surface - enzyme, membrane, etc. - suffer from difficulties in the stage of insertion into the subject tissue. The enzyme and/or membrane material can be destroyed or displaced using the insertion, to the detriment of the sensor device's effectiveness in subsequent use. This is almost unavoidable, as the friction between the tissue and the coating on the sensor device can be considerable --especially as the coating material is usually of a very delicate nature.

(3) It is very difficult to make sensor devices which are both small and effective. The fabrication of very small devices can be difficult, and it is also difficult to achieve the formation of reliable and stable coatings on them. For example, some devices proposed so far are little more than wires having an exposed tip which is the enzyme/membrane system -- and it is especially difficult to coat the tip of a thin wire.

For use, it has been proposed to implant electrodes in tissue through a cannula to avoid undesirable damage during the insertion stage, but this still involves some friction within the cannula. Attempts to enclose the whole device in a sheath of protective material which may contribute to the electrical function of the sensor electrode (e.g. a reference electrode) or as a strengthening or stiffening aid

have been proposed, but these tend to increase the size of the device and complicate provision of the necessary degree of exposure of enzyme-coated electrode for effective measuring activity.

The aim of our invention is to reduce the size of sensor implants to the practicable minimum compatible with robust mechanical integrity, and also to provide a compliant insert that reduces patient discomfort beyond that achieved to date.

We have now found that miniaturised devices which can overcome such disadvantages can be achieved by modifying the shape of the sensor device (especially one incorporating a wire electrode) by forming cavities in the otherwise smooth material of the sensor device and using these cavities to retain the enzyme. In this way, the enzyme can be retained within the profile of the sensor device and, as it no longer needs to protrude beyond the surface of the sensor device, it is thereby rendered less susceptible to being removed by friction or abrasion during insertion into tissue.

Thus according to our invention we provide new sensor devices comprising an enzyme electrode sensor in which active electrode material carries an enzyme, characterised in that the enzyme is retained within one or more cavities formed in the said electrode sensor.

By placing the cavity (or cavities) along the length of the electrode core, the enzyme therein can face laterally instead of being on a mechanically vulnerable wire tip.

The core of active electrode material may be made of any of the conventional conducting materials known for use in the art of sensor electrodes. Preferably it is a noble metal, for example gold or platinum, or an alloy of these with each other or one or more other elements. Preferably the material is platinum itself, but as platinum itself is relatively soft it can be hardened by alloying with a proportion of iridium.

The shape of the sensor device is most conveniently of a substantially circular cross-section, as is customarily the case when the active electrode material is a wire core, e.g. conventional drawn metal wire as available commercially, but may be be of any other cross-section if desired. The size of the core material is preferably in the range 50 to 150 μm. though larger or smaller sizes may be used if desired. Our aim is to use as thin a core as can be found to be practical, consistent with the requirement that its strength and integrity are not impaired by the size of the cavities.

As the purpose of the construction is for the sensor to present an enzyme-covered surface to its environment, it will normally be found that if any bare active electrode material is also exposed to the environment it will interfere with the measurements made at the surface beneath the enzyme. A bare surface of active electrode material can be tolerated if such interference does not occur, but the preferred form is that in which a core of the active electrode material is covered with a coating of insulating material to prevent bare active electrode material coming into contact with the environment media and the analyte to be detected and measured. Such an insulating material should be suitably durable, stable and resistant to the environment media, effectively sealed over the core of active electrode material, and - when intended for use in vivo - be suitably bio-compatible and harmless in use. Such materials are well known in the art.

Consequently, when the sensor to be used has such a coating of insulating material, the cavities required for our invention may be made in several ways. One way is for the insulation to be stripped off to expose a bare core of active electrode material and form the cavity into which the enzyme can then be placed. Alternatively, both the insulation and some of the core of active electrode material

can be removed by using an appropriate micro-machining technique, so that the insulation is removed and a cavity is also formed in the core of active electrode material itself. This latter procedure has the advantage that the fabrication is simplified and also that the cavities in the active electrode material allow for a greater surface area of the active electrode material to be exposed to enzyme and used to generate stronger signal outputs.

The cavities may be formed by conventional procedures, for example drilling, punching, grinding, boring, cutting, or any combination of these techniques, and the size and shape of the cavities may take any form which is considered most convenient and capable of retaining the desired enzyme in sufficient quantity. Likewise, the number of cavities may may be as large or small as desired, and will be determined to some degree by the size, shape and position of the cavities used in any particular instance. Our preference is for the cavities to be of a size up to about half of the overall thickness of the sensor material, so that the strength of the sensor is not unduly reduced. The optimum in any particular case may be readily determined by simple trial. The method used for forming the cavities may be a mechanical one, though that can be difficult on the micro-scale required; therefore we prefer to use an ion beam or laser method (commonly referred to a "micro-machining") as this is more easily used on the scale of size involved here. Thus a laser or ion beam can be used to etch, cut or bore into the material of the sensor to form the required cavities.

Examples of suitable shapes for the cavities include circular, oval, square, polygonal, cruciform, star-shaped and combinations of these. The cavities may regular or irregular in their, size, shape, number and distribution, though it is generally preferred (as being more convenient) to make all the cavities of substantially the same shape and

size. It is preferred that the form of the cavities should be chosen so that they can readily retain the enzyme; thus dish-shaped cavities may be less efficient if the enzyme is not strongly held, and cavities which are more like "pits" are usually to be preferred as they achieve a stronger hold on their enzyme contents.

Another useful cavity shape is a slot cut into the sensor in a substantially lengthways direction (i.e. in the direction of the axis of a wire electrode), as this can minimise the number of cavities to be made. The size of the slot (length and breadth) may be varied to suit particular needs and usually are not critical.

An especially useful form of cavity is one which passes completely through the core of electrode material, in effect forming a tunnel, open at both ends, running transversely to the general direction of the inner core. This allows the enzyme to be packed into this tunnel and the enzyme contents to be exposed to analyte and (in the case of an oxidase) also oxygen, as needed for reaction - thus giving very effective enzymatic action and consequent measurement efficiency. If desired the cavity may contain more than one enzyme, e.g. as laminate layers, so that a succession of reactions can be catalysed --- one enzyme acting on an analyte substrate to form a product which, in turn, is acted upon by the second enzyme to generate a further product which can then be satisfactorily detected and measured at the active electrode surface.

The enzyme may be used in conventional formulations and compositions, and placed in the cavities and retained therein by conventional coating methods and fixing methods. For example the enzyme may be applied as a composition which coats and fills the cavities (e.g. by dipping) followed by wiping or passing through a collar to remove the surplus and especially any on the main surface where it is not required. The enzyme can then be fixed in place by cross-linking, e.g.

by treatment with glutaraldehyde.

The enzyme may be any of the conventional enzymes used in sensor enzyme electrodes for electrochemical analysis, but we find oxidase or dehydrogenase enzymes are most useful. An especially useful example is glucose oxidase, which allows the device to be used for detection and measurement of glucose concentrations in tissues. Though we describe our invention with particular reference to glucose and glucose oxidase, however, it is not limited to this specific system and it is applicable to other substrate/enzyme systems, of which several are well known in the art for analytical purposes.

The sensor devices of our invention preferably also comprise coatings over the enzymes held within the cavities. Thus, additional layers of material may be deposited over the enzyme after that has been put into the cavities. Such over-coating layers may be composed of materials of appropriate permeability (simple or selective) to regulate the passage of components from a sample under examination to the enzyme and active electrode surface, or excluding or limiting access by materials which could interfere with the measurements. These materials are well known in the art and are usually in a thin form which serves as a permselective membrane, and may be applied by conventional means also well known in the art.

Examples of such materials include various polymers and polymer compositions, e.g. polyaryl ether sulphones and modified polyurethanes.

The electrodes of this invention may be used by any of the conventional procedures well known in the art, but of all the electrochemical procedures available we prefer to use an amperometric procedure with the active electrode material as the anode.

The sensor devices of our invention can be used <u>in vivo</u> or <u>in vitro</u>, and the mode of insertion into the sites for

making measurements are conventional. For in vivo sites, they may be inserted directly (transcutaneously into tissue) or through a cannula or even fine tubing. e.g. of nylon.

The advantages of the invention are especially in the way it provides new sensors which are small, light in weight, and potentially much more robust, flexible and suitable for implantation without attendant discomfort and other problems. Other advantages stem from the way in which the new sensors provide a different orientation and profile for the sensing surfaces - side-oriented micro-machined sensing surfaces - to the samples under examination,

The invention is illustrated but not limited by the accompanying drawings, which illustrate some forms which the improved electrodes can take.

These drawings are schematic and not drawn to scale, but are intended to show the principal features - in some cases emphasised by being out of scale.

In the drawings Figures 1 to 4 represent, in perspective view, various forms of sensor electrode devices of according to this invention and the type and disposition of cavities, and Figures 5 to 10 illustrate cross-sectional views through such sensor devices at the position at which the cavity is made.

In detail, all of Figures 1 to 4 show a thin platinum wire covered with insulation (1), with the said insulation covering the end (2) as well as the main body (1). The end (3) is adapted for continuation on and connection to the electrical measuring system (not shown).

In Figure 1, which shows the simplest form of the invention, the insulated wire (1) is pierced by a hole (4) to form a cavity (5) which is filled with an immobilised enzyme composition.

In Figure 2, there is shown a form in which the insulated wire (1) is pierced by a series of holes (6), each forming cavities filled with immobilised enzyme as in Figure

1. In Figure 3, there is shown a form in which the insulated wire (1) is pierced by a series of holes (7), each forming cavities filled with immobilised enzyme as in Figure 1 but of a shape different from those in Figure 2, i.e. cruciform instead of round.

In Figure 4, there is shown a form in which the insulated wire (1) is stripped of its insulation to form a slot (8) in the direction of the axis of the insulated wire (1), and the resulting slot is filled with immobilised enzyme; in this variant, the slot (8) has been made by cutting out the cover layer of insulation but without cutting into the core of platinum wire itself, but a further alternative (not shown) is that of cutting the slot into the core of platinum metal in addition to cutting away the outer layer of insulation.

All of Figures 5 to 10 illustrate cross-sectional views of alternative forms of the cavity through a thin platinum wire covered with insulation where the cavity is made.

In Figure 5, which shows the simplest form of the invention, the insulated wire comprises a core of thin platinum wire (1) covered by an outer layer of insulation (2) with a part of the insulation cut away to leave a cavity (3) filled with an immobilised enzyme composition.

In Figure 6, a cavity (3) has been bored through the inner platinum wire core, passing through it completely from one side to the other, and is filled with an immobilised enzyme composition. The enzyme-filled cavity (3) is coated at each end with a layer of a membrane coating (4) which acts to protect the enzyme and provide a chosen degree of selectivity or regulation of access of components of the surrounding medium to the enzyme.

In Figure 7, the cavity (3) has been bored through the inner platinum wire core, passing through it completely from one side to the other, and is filled with an immobilised enzyme composition as in Figure 6, except that two different

enzyme compositions (3A) and (3B) are used to fill the cavity (3). The enzyme contents are coated at each end with a layer of a membrane coating (4) as in Figure 6.

In Figure 8, the arrangement is essentially the same as for Figure 5 except that the cavity (3) is bored through the outer insulation layer (2) and also into the inner platinum wire core to form an inner pit in the platinum. This form has the advantage of having a larger surface area of the platinum exposed to enzyme and this produces greater response signals for measurement.

In Figure 9, the arrangement is essentially the same as for Figure 6 except that the cavity (3) bored through the outer insulation layer (2) and completely through the inner platinum wire core has not been given any coating (4), so that the enzyme composition contents of the cavity (3) are exposed directly to the surrounding media.

In Figure 10, the construction is that of Figure 6 but shows - with added arrows (9) - indication of flows of fluid past the membrane-covered apertures in the insulation. These flows may be fast or slow, large or small, and may be the same or different. One variant which is practicable is for the two flows (9A) and (9B) to be different -- with one (9B) being the sample medium, from which analyte components can diffuse in to the enzyme through the membrane, while the other flow (9B) can provide similar access by diffusion for necessary substrates for the enzyme system. For example, (9A) may provide the main source of glucose to a glucose oxidase system while (9B) may provide more of the oxygen necessary for the enzyme to function.

To fabricate the devices illustrated, the insulated platinum wire may be etched to remove the insulation layer and expose the platinum within, and subsequent polymerisation or other conventional techniques may be used to deposit the enzyme on the platinum surface. Covering the enzyme in the cavity can be achieved by conventional

techniques, for example application from solution by dipping. The thickness of the coating may vary, but should be thin enough to allow adequate diffusion of a desired analyte without unduly impeding the ability of the material contacting the enzyme to change frequently enough to give a satisfactory overall view of the rate of changes there may be in the medium surrounding the sensor device.

The etching and boring operations indicated above were carried out using a copper laser.

The thin platinum wire used is a commercially available one, as commonly used for making wire electrodes, since it is not necessary to use a special wire. If the coating of insulation on commercially available wire is not suitable for use in any particular intended environment, the coating as supplied can if necessary be removed and replaced by re-coating with a more suitable insulation or a coating of the preferred insulation material may be deposited over the one already on the commercial wire.

The method of fabrication according to this invention, and illustrated herein, enables insulated monopolar platinum wire electrodes having an outer diameter of 50 to 150 μm and a series of laser-drilled transaxial cavities (which we refer to as fenestrations) each approximately 30 μm in diameter for wire-shaft enzyme loading to be made without undue difficulty.

Using laser-etching of an insulated platinum wire of 25 to 50 μm outer diameter to remove insulation locally allows further miniaturisation and can create an ultra-fine working electrode surface on which thin layers of enzyme and barrier films can be deposited.

These provide embodiments provide protection for the active enzyme and have unique device geometry; configurations as illustrated provide side-oriented enzyme surfaces in micro-machined sensing surfaces designed to retain and protect them.

Construction as in Figure 6 in particular internalises the enzyme in the working electrode, giving a unique twin-surface enzyme with radial product diffusion into the surrounding platinum surface. The greater area of active platinum surface (i.e. surface exposed to the enzyme) can improve the efficiency with which the enzyme functions and so improve the measurements, for example in the linearity of response.

In use, the device can be inserted into tissue and, though the tip naturally must enter first, this entry does not damage the enzyme-containing areas of the surface. When the wire is pure platinum it may be too soft for direct insertion insertion to be easy, so subcutaneous implantation of such "soft" wires will be through narrow bore nylon tubes. Alternatively, direct insertion can be made easier by using a platinum/iridium alloy wire, which is more rigid.

For determination of analytes, the procedures used were conventional ones and not described in detail here as they are well known in the art and do not involve any departures from the usual ones.

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Claims:

1. A sensor device comprising a wire electrode sensor carrying an enzyme, the wire electrode having a plurality of cavities formed along the length of the electrode which retain the enzyme.

2. The sensor device of claim 1, wherein the cavities are circular, oval, square, polygonal, cruciform, star-shaped or combinations thereof.

3. The sensor device of claim 1 or claim 2, wherein the wire electrode sensor is formed from a noble metal.

4. The sensor device of claim 3, wherein the noble metal is gold, platinum, or an alloy thereof.

5. The sensor device of claim 4, wherein the alloy is platinum-iridium.

6. The sensor device of any one of the preceding claims, wherein the wire has an outer diameter of 50 to 150μm.

7. The sensor device of any one of the preceding claims, wherein the enzyme is an oxidase or dehydrogenase enzyme.

8. The sensor device of claim 7, wherein the enzyme is glucose oxidase.

9. The sensor device of any one of the preceding claims, wherein the enzyme is retained in the cavities by cross-linking.

10. The sensor device of claim 9, wherein the enzyme is cross-linked using glutaraldehyde.

11. The sensor device of claim 10, wherein the sensor device is used for the measurement of glucose concentrations in tissues.

12. The sensor device of any one of the preceding claims, wherein the electrode further comprises a coating over the electrode and enzyme present in the cavities.

13. The sensor device of claim 12, wherein the coating regulates the passage of components from a sample under examination to the enzyme and active electrode surface.

14. The sensor device of claim 13, wherein the coating is a polymer or polymer composition.

15. The sensor device of claim 14, wherein the polymer composition is a polyaryl ether sulphone or a modified polyurethane.

16. The sensor device of any one of the preceding claims, wherein the cavities are produced by micromachining with an ion beam or a laser.

17. The sensor device of any one of the preceding claims, wherein the surface or the wire electrode is covered by a coating of insulating material.

18. The sensor device of claim 17, wherein the cavities are produced by removing insulating material from the electrode.

19. Use of a sensor device of any one of the preceding claims for determining or monitoring an analyte.

20. The use of claim 19, wherein the analyte is glucose.

21. The use of claim 19 or claim 20, wherein the analyte is determined or monitored in an amperometric procedure.

22. The use of any one of claims 19 to 21, wherein analyte is determined or monitored by inserting the electrode transcutaneously through cannula.

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Published:
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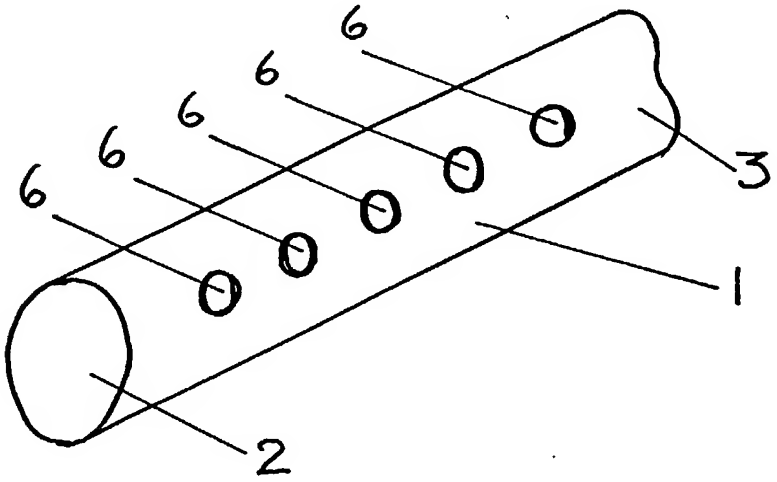
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE



(57) Abstract: Sensor devices with an enzyme retained in one or more cavities formed in active electrode core, having the cavities along the length of the electrode core so that the enzyme faces laterally instead of towards or at the mechanically vulnerable tip. This improves effectiveness and ease of use. They are especially wire electrodes, preferably of platinum, and the cavities may be formed as holes cut in the covering insulation on a wire or cut deeper and into the electrode core material itself, e.g. by laser-drilling. One preferred cavity form is a lateral slot and another is a "tunnel" hole cut through the core to expose enzyme at both ends, and the enzyme can be covered by a porous or perm-selective membrane. The sensors are useful for measuring of analytes amperometrically in biological media, and especially glucose on blood/serum using glucose oxidase as the enzyme.

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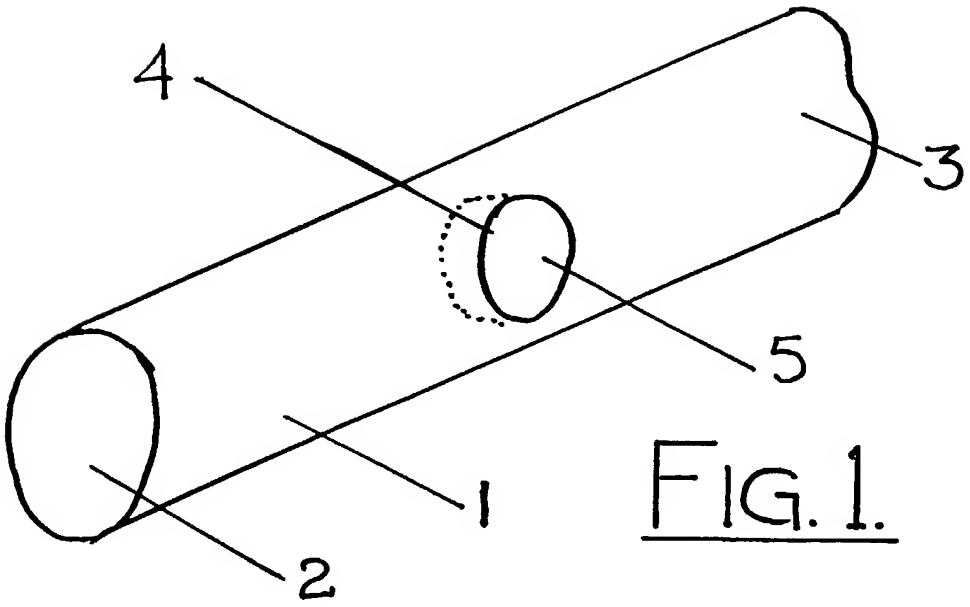

FIG. 1.

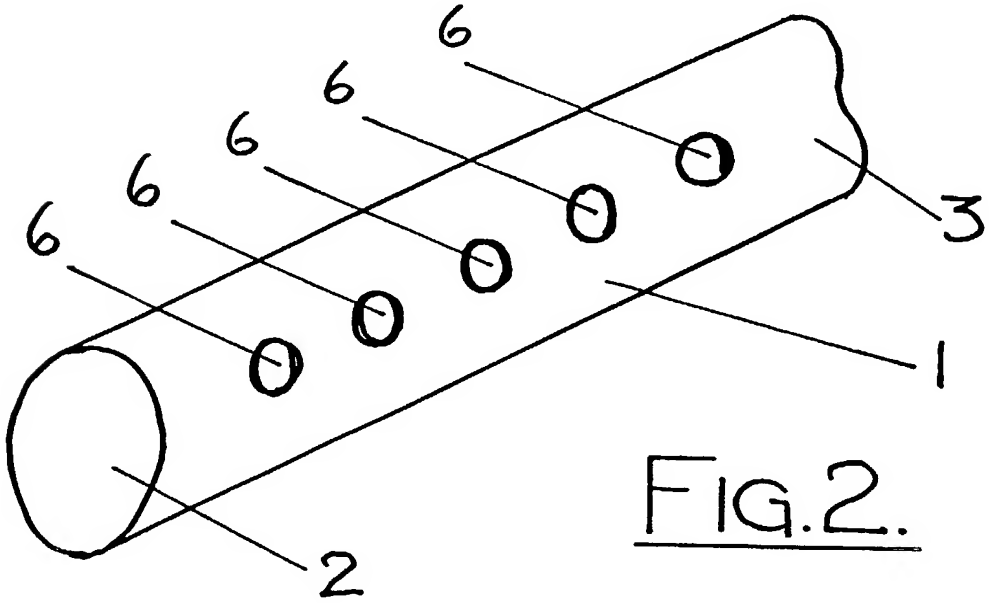

FIG. 2.

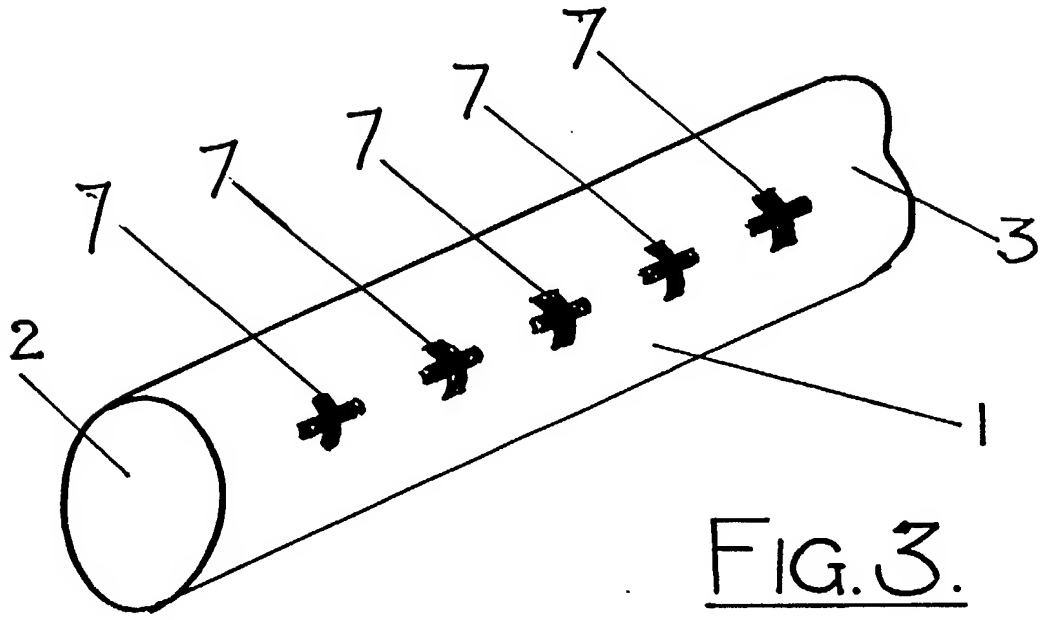

FIG. 3.

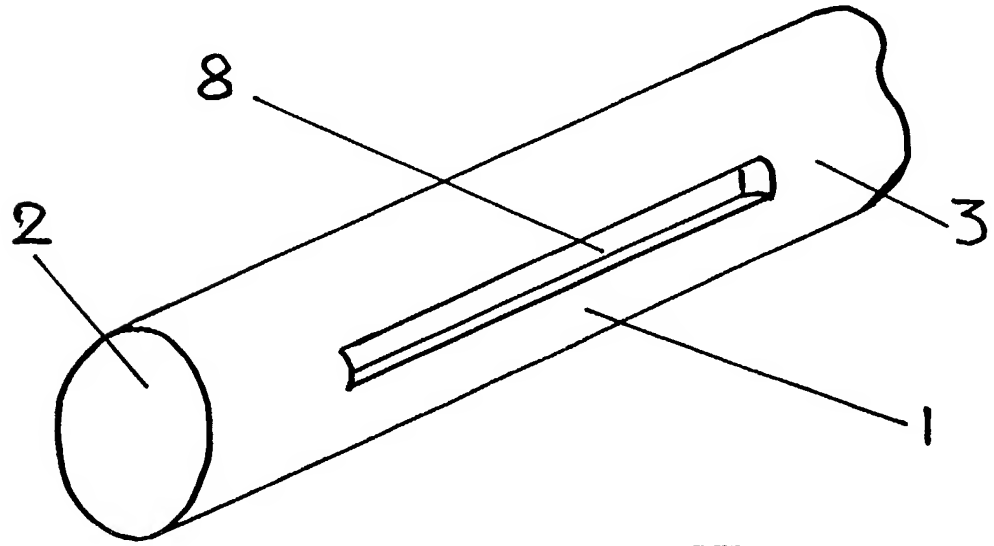

FIG. 4.

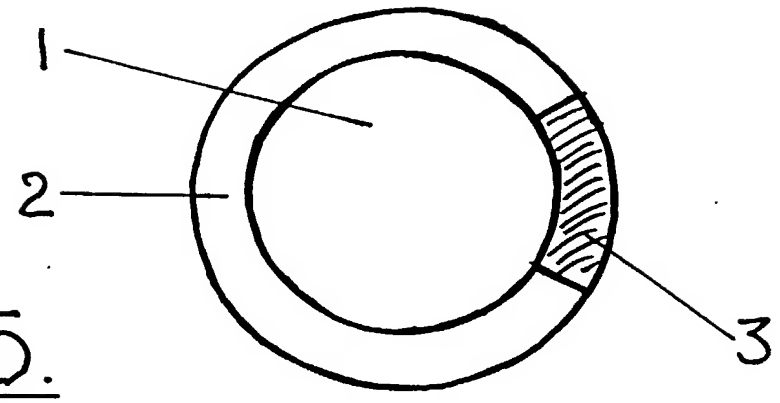

FIG. 5.

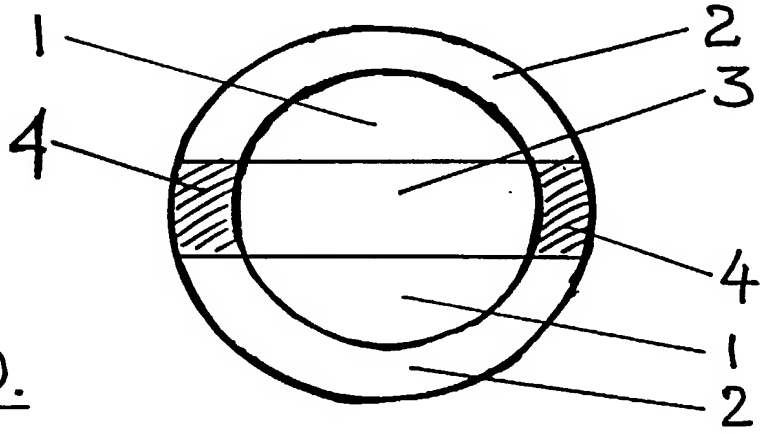

FIG. 6.

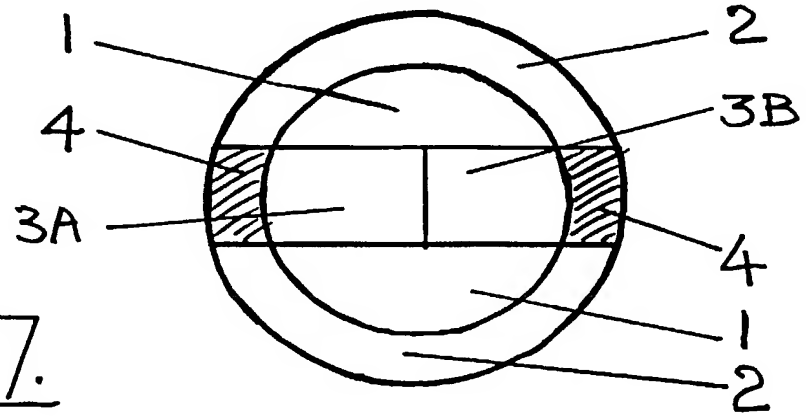

FIG. 7.

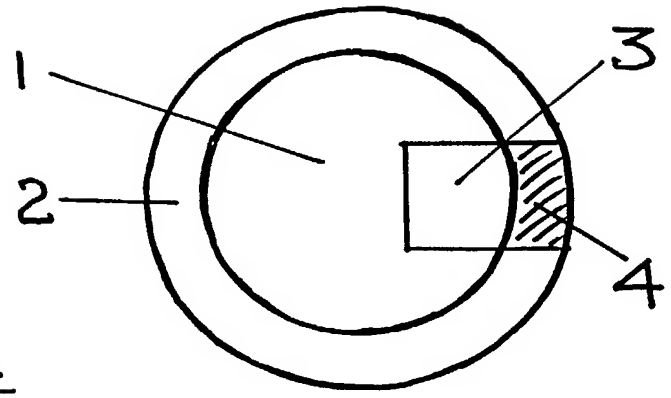

FIG. 8.

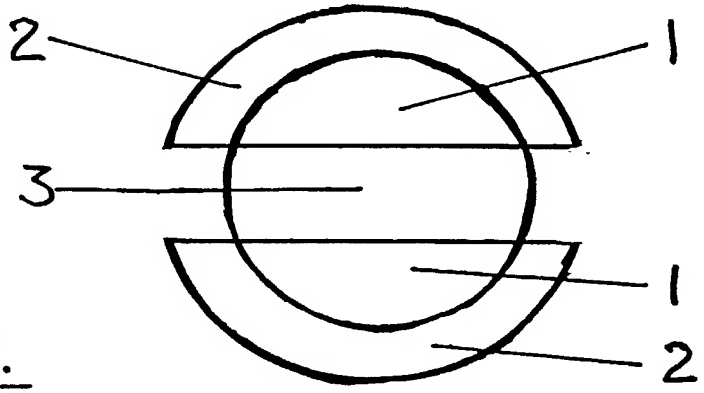

FIG. 9.

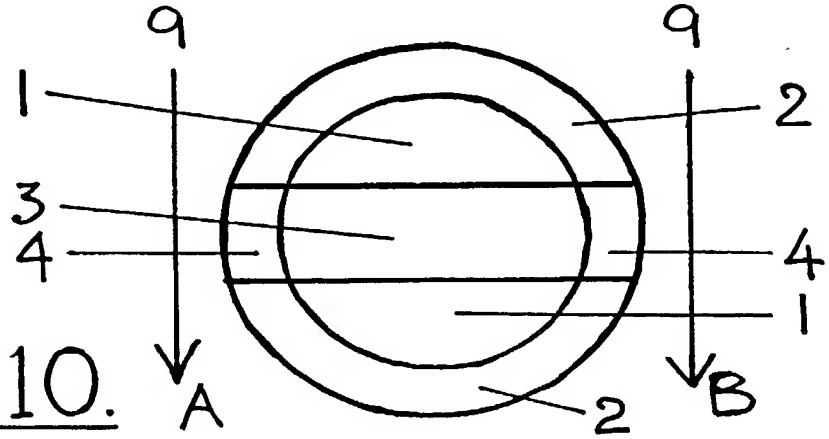

FIG. 10.

DECLARATION, POWER OF ATTORNEY AND POWER TO INSPECT

As a below named inventor, I hereby declare:

that my residence, post office address and citizenship are as stated below next to my name;

that I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the invention entitled: **SENSOR DEVICES AND ANALYTICAL METHODS FOR THEIR USE** the specification of which [check one(s) applicable]

- X was filed 8 August 2000 as International Patent Application No. PCT/GB00/03054, on which U.S. National Stage Application No. 10/049,326 is based; and/or
- ___ was amended by Amendment filed _____ (if applicable); and/or
- ___ is attached to this Declaration, Power of Attorney and Power to Inspect;

that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and

that I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Rule 56(a) [37 C.F.R. §1.56(a)].

CLAIM UNDER 35 U.S.C. §119: I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application of which priority is claimed:

Prior Foreign Application(s) Appln No.	Country	Filing Date Day-Mon-Year	Priority Claimed Yes - No
9918839.3	Great Britain	11-08-1999	Yes

(2)

POWER OF ATTORNEY: As inventor, I hereby appoint **DANN, DORFMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, Pennsylvania, and the following individual(s) as my attorneys or agents with full power of substitution to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith: **Patrick J. Hagan, Reg. No. 27,643 and Kathleen D. Rigaut, Ph.D., Reg. 43,047.**

POWER TO INSPECT: I hereby give **DANN, DORFMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, Pennsylvania or its duly accredited representatives power to inspect and obtain copies of the papers on file relating to this application.

SEND CORRESPONDENCE TO: **CUSTOMER NUMBER 000110**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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